Transcriptomic approach to identify genes involved in pathogenicity of *Ehrlichia ruminantium*

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INTRODUCTION

- ✓ *Ehrlichia ruminantium* (*ER*): agent of heartwater, a tropical fatal disease of ruminants
- ✓ Lack of efficient vaccines due to high genetic diversity
- ✓ Genomic sequence for 3 virulent strains: Gardel, Senegal & Welgevonden

OBJECTIVES

✓ To understand the mechanisms of virulence of *ER* by differential gene expression: virulent vs. attenuated strains and at different stages of development

PROTOCOL FOR GENE EXPRESSION STUDY

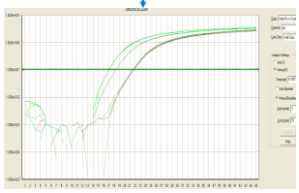
Gardel & Senegal virulent and attenuated strains at different times post infection

TC flask with *ER* infected bovine endothelial cells

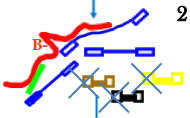
DNA Extraction from 1/10 of sample

Extraction of total RNA from 9/10 of sample

RT & Amplification



Quantification of *ER* by q-PCR targeting *map1* & *pCS20*



2 Selective captures of *ER* transcripts

Micro-arrays hybridization

Identification of genes differentially expressed between

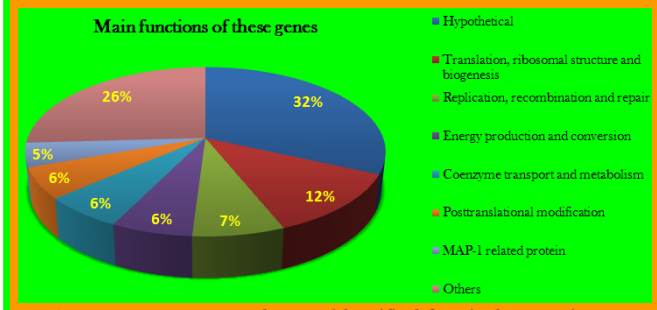
Virulent & attenuated strains

Stages of development

Validation of identified genes by q-RTPCR

MICROARRAY RESULTS BETWEEN VIRULENT & ATTENUATED GARDEL STRAINS

25% (239/950) of *ER* identified genes are differently expressed

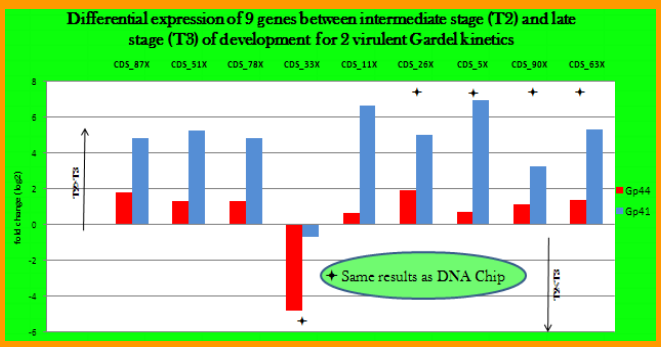


Among over-expressed genes identified for virulent strains, some genes seem to be involved in evasion of the host cell immune response

RESULTS BETWEEN DIFFERENT STAGES OF DEVELOPMENT FOR GARDEL VIRULENT

5% (54/950) of *ER* genes identified by µarrays are differentially expressed between intermediate T2 & late T3 stages of development

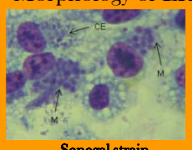
Validation of gene overexpression for 14 genes by q-RTPCR



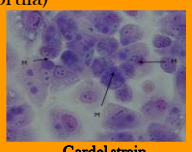
- ➢ At T2, 8 over-expressed genes coding for proteins involved in metabolism (3 CDS), in the transport & exchange of nutrients (4 CDS) & in resistance to oxydative stress (1 CDS)
- ➢ At T3, one single over-expressed gene coding for transcription factor & known as inducer of virulence in *Salmonella typhimurium*
- ➢ Further µarrays analysis are ongoing to understand the discrepancies between q-RTPCR and µarrays results for 5 genes

SENEGAL & GARDEL STRAINS: WIDELY DIFFERENT

- ➢ Morphology of *ER* colonies (Morula)

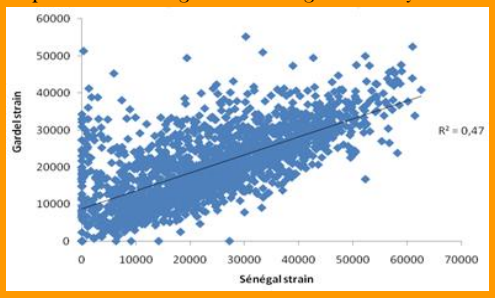


Senegal strain



Gardel strain

- ➢ Comparison of Senegal & Gardel genomes by micro-arrays



No antigenic protection between Senegal & Gardel strains

- ➢ Attenuation after **15 passages** & **200 passages** for Senegal and Gardel respectively

CONCLUSION

Some genes coding for proteins involved in the virulence & in the development of the bacteria were identified for Gardel strain. The gene expression results will be compared to the results of *ER* genome sequencing & proteomic projects in order to understand the behavior of *ER*. The differential gene expression of Senegal strain, virulent & attenuated forms, will be studied in order to identify other mechanisms of attenuation.

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